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## (54) TRANSCRIPTIONAL REGULATORY SEQUENCE AND USE THEREOF

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a transcriptional regulatory DNA.
SOLUTION: A genom DNA region containing a sequence of about 4.0 kb on the upstream of a MEGSIN gene is separated and the base sequence is determined. A region positively regulating the transcription region is specified in the genom DNA region. An AP-1 bond motif in the region is found to exhibit an activity to positively regulate the transcription activity.

The transcription activity is considerably lowered by deleting the sequence or transducing a variation from/into the sequence by a site-specific mutagenesis. The transcriptional regulatory DNA is useful as a transcriptional regulatory sequence specific to mesangium cell. The DNA is useful also as a transcriptional factor for controlling the expression of MEGSIS gene and the screening of medicines.

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## DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to DNA which participates in the transcriptional control of a gene. DNA of this invention is applicable to fields, such as gene therapy.
[0002]
[Description of the Prior Art] No less than 60 trillion various cells in the living body have the same genomic DNA intrinsically. For the normal physiological function, such gene expression is strictly controlled by cell lineage and the signal which a cell receives. Therefore, it is very important to solve the gene specifically discovered with each cell type.
[0003] The mesangium (mesangium) is an organization which becomes the heart which is located in the leaflet core of the capillary tube loop of mesangium, and ties up each leaflet. The mesangium is covered with glomerular basement membrane and consists of quality of an intangible asset (mesangium substrate: mesangial matrix) which is following the inner clear layer in the glomerular basement membrane which serves as a cell (mesangial cell: mesangial cell) separated by the endothelial cell from three layers with the capillary tube cavity.
[0004] It is thought that having played the central role is known by the structure of mesangium and maintenance of a function, and mesangial cells are the main factors in the onset of glomerular diseases, such as glomerulonephritis and glomerulosclerosis. And the mesangial cell serves as a target of a failure in various nephritides. For example, growth of a mesangial cell and are recording of an extracellular mesangium substrate It considers as the first process in which the glomerulosclerosis is brought to the patient who has the chronic glomerulonephritis which is the two major causes of terminal renal failure, and various glomerulus failures like diabetic nephropathy. [D. Schlondorff, Kidney Int., and 49, 1583-1585; (1996) R.B.

Sterzel and H.D.Rupprecht, Glomerular mesangial cells.In: Neilson, E. G.Couser, W.G.eds., "Immunologic Renal Diseases", Philadelphia: Lippincott-Raven, pp595-626 (1997)]. Therefore, it is effective in the therapy of the disease relevant to investigation of the cause of the disease relevant to a break through of the biological property of a mesangial cell, and a mesangial cell, as a result a mesangial cell, a diagnosis, etc. to find out the gene specifically discovered by the mesangial cell, and to clarify the regulatory mechanism of the manifestation.

[0005] By the way, in large-scale DNA sequencing and database analysis, this invention person isolated the gene named MEGSIN as a gene especially discovered strongly by the mesangial cell, and determined all the base sequences. And the array of the new protein (Homo sapiens MEGSIN) with which the overall-length cDNA clone of MEGSIN consists of 380 amino acid which carries out a code was determined. Furthermore, the place which performed amino acid homology retrieval by the FASTA program using the Swiss Prot database, Homo sapiens MEGSIN SERPIN (serine protease inhibitor) super family [R.Carrell et al., Trends Biochem.Sci., 10, and 20; (1985) R.Carrell et al. and Cold Spring Harbor Symp.Quant.Biol., 52 527; (1987) E.K. O.Kruithof etal. and Blood, 86 4007; (1995) J. Potempa et al., J.Biol.Chem., 269, and 15957; (1994) E.Remold-O'Donnell, FEBSLett., 315, and 105 (1993)] That it is the protein which belongs It found out. [T.Miyata etal., J.Clin.Invest., 120, 828-836;(1998) WO 99/15652].

[0006] By Homo sapiens fibrocyte, the smooth muscle cell, the endothelial cell, and keratinocyte, Homo sapiens MEGSIN has a weak manifestation and is especially discovered strongly by the mesangial cell (that is, Homo sapiens MEGSIN gene expression has singularity in a mesangial cell). Moreover, when a IgA glomerulonephritis patient, a diabetic nephropathy patient, and healthy people compare the amount of manifestations of MEGSIN under kidney tissue, in a IgA glomerulonephritis patient or a diabetic nephropathy patient, the amount of manifestations has much MEGSIN intentionally. [D.Suzuki et al., J.Am.Soc.Nephrol.10, 2606-2613;(1999) WO 00/57189]. Moreover, in the mesangial-proliferative-glomerulonephritis model using Latt, lifting of this amount of gene expression was accepted (WO 01/48019). Moreover, by the transgenic mouse of MEGSIN, since self-possessed sthenia of the immune complex which changes from an immunoglobulin or complement to the hyperplasia of the Tsuguaki cell proliferation which makes a mesangial cell a subject, and a mesangium substrate, and a list is accepted and the symptom of typical mesangial proliferative glomerulonephritis is presented, sthenia of MEGSIN gene expression is considered to contribute to mesangial proliferative glomerulonephritis (WO 01/24628).

[0007] Thus, since MEGSIN gene expression might be participating in kidney disease deeply, it clarified the actual condition of a MEGSIN gene expression

controlling mechanism, and the diagnosis to the hereditary disease caused by a break through and its variation of Homo sapiens's MEGSIN function in the living body, offer of a transcriptional control array useful for a therapy, or a kidney mesangial cell was expected to offer the transcriptional control array discovered specifically.

[0008] It is thought that it branched from ancestor protein common to an evolution top on the other hand since the SERPIN super family to which Homo sapiens MEGSIN belongs had high homology in both primary-structure tops. that is The number of variation amino acid on an amino acid sequence [K. Suzuki et al. and Tanpakushitsu Kakusan Koso, 34, and 949-962 (1989)] Phylogenetic tree produced based on chromosome gene structure [J.J.Bao et al., Biochem., 26, and 7755] (1987) The result of analysis, It is shown that the SERPIN super family has evolved over 5 million or more with various high vertebrates. However, the MEGSIN gene is very characteristic of a glomerulus mesangial cell at the point of being specifically discovered.

[0009] Moreover, an ion channel and the gene concerning transport recently it is reported that it is specifically discovered into the kidney [S.J.Lolait et al., Nature, 357, and 336-339 (1992); Y.Kanai et al. and J.Clin.Invest., 93 and 397-404; (1994) S.Uchida et al. and J. Biol.Chem., 268, and 3821-3824 (1993); S.Adachi et al. and J.Biol.Chem., 269 and 17677-17683(1994); K.Fushimi et al., Nature, 361, and 549-552; (1993) G.Gamba et al., J.Biol.Chem., 269, 17713-17722 (1994)]. However, localization of these genes is carried out to the renal tubule epithelial cell, and they are not discovered by the glomerulus mesangial cell. therefore, the thing for which the transcription factor which participates in the transcriptional control array of a MEGSIN gene and the imprint of a MEGSIN gene is solved — a cell type — the important information about an anaclitic gene expression device can be acquired. The information acquired by doing still in this way is applicable also to the target cell in molecular genetics or transgenics.

[0010] this invention persons are considering the DNA array in connection with the transcriptional activity of the near [ upstream region abbreviation-1.2kbp ] from the transcription initiation site of a MEGSIN gene until now (WO 00/43528). However, it is not suggested at all whether the array found out all over the promoterregion of a MEGSIN gene in this invention is participating in imprint induction.

[0011]

[Problem(s) to be Solved by the Invention] This invention makes it a technical problem to offer DNA including a transcriptional control array, and its utilization.

[0012]

[Means for Solving the Problem] this invention persons are an upper (5' side) abbreviation of a MEGSIN gene which was not isolated until now in order to

solve a MEGSIN gene expression regulatory mechanism. The base sequence of the genomic DNA containing 4.0 kb was newly determined. Moreover, this invention persons determined Homo sapiens's MEGSIN mRNA transcription initiation site by the primer extension method using mRNA prepared from the culture Homo sapiens mesangial cell. After the primer extension method exists down-stream from a transcription initiation site, makes a primer RNA imprinted and a single stranded DNA which has a complementarity and carries out the label of the five prime end, it is an approach the magnitude of the fragment which was made to carry out hybridization to RNA, was expanded to the transcription initiation site using reverse transcriptase, and was obtained determines a transcription initiation site as compared with the ladder of the sequence of the genomic DNA using the same primer. It succeeded in this invention persons identifying transcription initiation sites with a main Homo sapiens MEGSIN gene by this approach.

[0013] It became clear that the TATA box (TATA box) which is a promotor array existed in the part of upper abbreviation 35bp of the identified transcription initiation site, and the transcriptional control array which can serve as an imprint control section of AP-1 and oct-1 grade in the upstream existed further. Upstream region abbreviation of a MEGSIN gene including this transcriptional control array The luciferase reporter structure was produced using DNA to which deletion of the field of 4.0 kb was carried out gradually, and the promotor activity of each structure was measured using the Homo sapiens mesangial cell and the epidermoid-carcinoma cell strain A431. Consequently, it traced that the array which just controls an imprint existed in the field of -240--72 from the transcription initiation site of a Homo sapiens MEGSIN gene.

[0014] In order to identify further the array which participates in transcriptional control in a detail, as a result of producing the reporter structure which includes MEGSIN promoterregion in 10 bp unit from -179-+130 to -72-+130 and examining the transcriptional activity, it became clear that transcriptional activity decreased sharply between -121-+130 and -99-+130. - the place which searched the motif between 121--99 -- AP-1, oct-1, and Brn-2/TCF-11 etc. -- the array of a high score was found. Base sequence including the array concerned As a result of producing the construct to which deletion of the 55bp (-85--139) was carried out and examining transcriptional activity, in the construct (-85 - -139 deletion structure) to which deletion of this 55 bp was carried out, transcriptional activity fell thoroughly.

[0015] AP-1 contained during this array, and oct-1 -- or -- TCF-11 in order to identify the array which has a role in transcriptional control inside -- AP-1 and oct-1 -- or -- TCF-11 The construct including each variation was produced and such transcriptional activity was examined. Consequently, by

AP-1 mutant, lowering of about 50% of transcriptional activity was seen to fluctuation not being looked at by transcriptional activity in oct-1 mutant. Furthermore, when examination same about the construct to which deletion only of the part of an AP-1 joint motif was carried out was performed, it became clear that transcriptional activity fell about 50% like a mutant. Thus, this invention persons are AP-1 junction sequences which exist in upper-120--112 from the transcription initiation site of a Homo sapiens MEGSIN gene. CTGATTCAC It has the important function, in order that included DNA may just control transcriptional activity, and it was proved that a role essential to Homo sapiens MEGSIN gene expression control was played.

[0016] this invention persons succeeded in identifying the new DNA array which participates in transcriptional control in the promoterregion of a MEGSIN gene as mentioned above. If DNA including this array is used, it will become possible to develop the expression vector which makes a foreign gene discover in specific cells, such as a mesangial cell, for example. Moreover, the drugs which adjust the manifestation of a MEGSIN gene etc. can also be obtained through screening of the compound which adjusts association with this DNA and transcription factor. As mentioned above, it is known that sthenia of MEGSIN gene expression will cause the nephropathy of mesangial cell fecundity, and the utilization as a remedy [ as opposed to kidney disease in the drugs which can control the manifestation of MEGSIN ] is expected.

[0017] This invention relates to DNA including a transcriptional control array, and its utilization. More specifically (1) following (a) or DNA given in (b), (a) -- array number: -- isolated DNA including the base sequence which at least 15 bases which contain CTGATTCAC of the base sequence of a publication in 1 followed -- In the base sequence which at least 15 bases which contain CTGATTCAC of the base sequence of a publication in 1 followed (b) -- array number: -- It is isolated DNA which includes a permutation, deletion, and/or the inserted base sequence for 1 or two or more bases other than this CTGATTCAC. Include the process which inserts in imprint regulatory region DNA which has the activity which just controls an imprint, and DNA given in (2) and (1). From the approach of just controlling an imprint, and the imprint regulatory region which contains DNA of a publication in (3) and (1) The approach including the process which makes a CTGATTCAC array suffer a loss of controlling an imprint to negative, It is the detection approach of the compound combined with DNA given in (4) and (1). (a) The process which contacts this DNA to a sample compound, the process which detects association with (b) this DNA and this sample compound, They are the ****** approach and the screening approach of the compound combined with DNA given in (5) and (1). (a) The process which contacts this DNA to a

sample compound, the process which detects association with (b) this DNA and this sample compound, (c) It is the approach of evaluating the effectiveness of an approach including the process which chooses the compound combined with this DNA, and the compound exerted on association with DNA given in (6) and (1), and AP-1. (a) The process at which this DNA and AP-1 are contacted under existence of the sample containing a sample compound, (b) -- this DNA -- this -- this [ association / which detect association with AP-1 / the process and (c) this association ] DNA under the nonexistence of a sample compound -- this -- by comparing with association with AP-1 They are an approach including the process which evaluates the effectiveness of a sample compound, and the screening approach of a compound of adjusting association with DNA of a publication, and AP-1 to (7) and (1). (a) The process at which this DNA and AP-1 are contacted under existence of the sample containing a sample compound, (b) -- this DNA -- this -- this [ association / which detect association with AP-1 / the process and (c) this association ] DNA under the nonexistence of a sample compound -- this -- by comparing with association with AP-1 the process and (d) this DNA which evaluate the effectiveness of a sample compound -- this -- the process which chooses the compound which adjusts association with AP-1 -- The ****** approach, the recombinant DNA by which the gene was functionally connected with the lower stream of a river of DNA given in (8) and (1), (9) It is the approach of evaluating the effectiveness of a sample compound exerted on transcriptional activity. (a) by comparing with the manifestation of DNA of a publication the process which makes (8) discover DNA of a publication under existence of the sample containing a sample compound, the process which detects (b) this manifestation, and (c) this manifestation (8) under the nonexistence of a sample compound They are an approach including the process which evaluates the effectiveness of a sample compound, and the approach of screening the compound which adjusts (10) transcriptional activity. (a) by comparing with the manifestation of DNA of a publication the process which makes (8) discover DNA of a publication under existence of the sample containing a sample compound, the process which detects (b) this manifestation, and (c) this manifestation (8) under the nonexistence of a sample compound The process which evaluates the effectiveness of a sample compound, the process which chooses the compound which adjusts (d) this manifestation, (The ****** approach, (11) and (5), 7) or the transcriptional control agent containing the compound in which it is chosen as (10) by the approach of a publication, and deals, It is the transcriptional control agent and the transcriptional control agent of (13) MEGSIN gene which make DNA of a publication an active principle (12) and (1). (11) Or it is related with the mesangial cell growth modifier which contains DNA of a

publication in (a transcriptional control agent given in (12), (14) and (5), 7), the compound which it is chosen by (10) by the approach of a publication and is sold to it, or (1).

[0018]

[Embodiment of the Invention] This invention is isolated DNA including the base sequence by which at least 15 bases of the base sequence of a publication followed array number:1, and offers DNA which contains CTGATTCAC (array number: 2) in the base sequence which this continued. this invention persons found out having the activity by which the CTGATTCAC array which exists in the upstream region of the transcription initiation site of a Homo sapiens MEGSIN gene just controls the imprint of the gene connected down-stream. DNA of this invention including this array is useful as a transcriptional control element. DNA of this invention is still more useful to the joint assay of a transcription factor.

[0019] DNA of this invention may be natural DNA or a natural synthetic DNA. Income of the natural DNA can be carried out by screening of a genomic DNA library from genomic DNA, such as mammalian of Homo sapiens and others, by using the genome DNA fragment of the upstream region of MEGSIN cDNA or a MEGSIN gene etc. as a probe. Moreover, the genomic DNA of Homo sapiens or other animals can be isolated by performing polymerase chain reaction (PCR) to mold using the primer created based on the array of MEGSIN cDNA or genomic DNA. The MEGSIN gene which exists in a DNA fragment, and DNA of the upstream region can be acquired according to JP,6-181767,A or reference (The Journal of Immunology 155, 2477-2486; (1995) Proc.Natl.Acad.Sci.USA 92, and 3561-3565 (1995)). If it is a synthetic DNA, it can manufacture, for example according to the conventional method using the chemosynthesis of the nucleic acid of the phospho friend DAIDO method [Mattencci, M.D.& Caruthers, M.H.J., Am.Chem.Soc.103, and 3185] (1981) or a phosphite triester method [Hunkapiller, M.et al., Nature 310, and 105] (1984). The nucleotide with which DNA of this invention was embellished may be contained. Moreover, in this invention, a single stranded DNA and double stranded DNA are contained in DNA. That is, this invention offers the single stranded DNA which consists of a chain of either double stranded DNA including Above CTGATTCAC, and this double stranded DNA. Double stranded DNA can detect association of a transcription factor, or it can be used for it as some promotors. The single stranded DNA is useful in the transcription inhibition for example, by Mie chain DNA formation etc. In addition, double stranded DNA may have the single strand part in the location of an end or others. DNA of this invention is double stranded DNA preferably. 15 or more nucleotides of 20 or more nucleotides of 25 or more nucleotides of chain length of DNA of this invention are 30 or more nucleotides still more preferably more preferably,

for example. Moreover, 3000 or less nucleotides of 1000 or less nucleotides of 500 or less nucleotides of chain length of DNA of this invention are 200 or less nucleotides still more preferably more preferably, for example.
[0020] As [ indicate /, for example / as DNA of this invention / by the example ] Homo sapiens MEGSIN gene -4021-+130, -2542-+130, -1874-+130, -1451-+130, -1052-+130, -834-+130, and -504-+130 -- or -- The base sequence of -240-+130 Included DNA is mentioned (refer to the; example showing the location which set +1 and the base in front of that to -1 for the transcription initiation site for a figure). -179-+130, -169-+130, -159-+130, -149-+130, -139-+130, and -129-+130 -- or -- DNA including the base sequence of -121-+130 etc. is mentioned. [ moreover, ] Moreover, DNA which does not include the array by which a lower stream of a river is imprinted from the transcription initiation site of a MEGSIN gene as DNA of this invention is more suitable. Such DNA is useful in order to control the imprint of a down-stream gene combining other genes or the imprint unit designed artificially. Moreover, such DNA can also be used for transcription factor joint assay. Specifically as DNA of this invention, it is a Homo sapiens MEGSIN gene preferably. Do not include the base sequence to -101-+86. more -- desirable -- the base sequence to -103-+86 is not included -- more -- desirable -- the base sequence to -105-+86 is not included -- more -- desirable -- the base sequence to -107-+86 is not included -- more -- desirable -- DNA which does not include the base sequence to -109-+86 is mentioned.
[0021] Moreover, DNA of this invention is DNA containing CTGATTCAC (array number: 2), and is in the 1st of a base sequence given in array number:1, for example. Isolated DNA which consists of a base sequence which at least 15 bases in the base sequence to the 3921st (-102; location from a transcription initiation site) followed is contained. DNA of this invention is DNA including a CTGATTCAC array still more preferably. To array number:1 from the 1st of the base sequence of a publication to the 3919th (-104) It is isolated DNA which consists of a base sequence which at least 15 bases of the base sequences from the 1st to the 3913rd (-110) followed more preferably [ it is more desirable, is more desirable from the 1st to the 3917th (-106), and ] from the 1st to the 3915th (-108). Moreover, this invention offers the recombinant DNA which other DNA combined with either of the DNA which consists of these partial arrays of array number:1, or both ends.
[0022] Moreover, in the base sequence which at least 15 bases which contain CTGATTCAC in the base sequence of a publication in array number:1 followed, this invention is isolated DNA which includes a permutation, deletion, and/or the inserted base sequence for 1 or two or more bases other than this CTGATTCAC, and offers DNA which has the

activity which just controls an imprint. In DNA including the base sequence which at least 15 bases which contain CTGATTCAC of the base sequence of a publication in array number:1 of above-mentioned this invention followed as such DNA, for example, it is DNA which consists 1 or two or more bases other than this CTGATTCAC of a permutation, deletion, and/or an inserted base sequence, and DNA which has the activity which just controls an imprint is mentioned. For example, DNA containing the fragment of the promoterregion of a Homo sapiens MEGSIN gene can change an array for 1 or two or more bases a permutation, insertion, and/or by carrying out deletion into parts other than CTGATTCAC of the promoterregion. It is such DNA and DNA which has the activity which just controls an imprint is contained in DNA of this invention. For example, in the example, even if this invention persons changed oct-1 junction sequence located near the CTGATTCAC in the promotor of a Homo sapiens MEGSIN gene (-111 - -110 part), they showed that transcriptional activity was maintained. Moreover, a transcription initiation site is received. Even if it carried out deletion of the DNA of the field to -4021--121, it did not have significant effect on transcriptional activity. Therefore, these base sequences can be changed suitably, without making transcriptional activity lose, when these arrays are included in DNA. Although a permutation, deletion, and/or the number of bases to insert can be suitably adjusted in a base sequence It is under [ base sequence / of the continuous arbitration which consists of 15 bases which contain CTGATTCAC of the base sequence of a publication in 1 ] setting. For example, the array number contained among the target DNA : Considering as one base most preferably is still more preferably desirable [ still more preferably / 2 bases ] three or less bases four or less bases five or less bases preferably.

[0023] DNA of this invention may be combined with other DNA. DNA of arbitration is mentioned as other DNA, for example, various promotor arrays are included. Moreover, genes, such as DNA which carries out the code of the protein, can be connected with the imprint field of the lower stream of a river (3' side of the chain containing CTGATTCAC) of DNA of this invention. Moreover, as for DNA of this invention, multiple copy **** DNA is contained in CTGATTCAC. For example, CTGATTCAC may be included two copies, three copies, or more than it.

[0024] DNA of this invention can be used in order to detect the compound combined with this DNA. This invention is the approach of detecting the compound combined with DNA of this invention, and relates to an approach including the process which contacts the sample containing the (a) sample compound to DNA of this invention, and the process which detects association with (b) this DNA and this compound. Moreover, if this detection approach is used, it is possible to screen the compound combined with DNA

of this invention. That is, this invention is the approach of screening the compound combined with DNA of this invention, and relates to an approach including the process which contacts the sample containing the (a) sample compound to DNA of this invention, the process which detects association with (b) this DNA and this compound, and the process which chooses the compound combined with (c) this DNA. Especially this approach is used in order to detect or screen the protein combined with DNA of this invention. A transcription factor can be mentioned especially as protein combined with DNA of this invention. It combines with DNA directly or indirectly, and a "transcription factor" means the protein which adjusts the gene expression of the lower stream of a river of this DNA to forward or negative. Although it is not indispensable, protein indispensable to an imprint, the protein which adjusts imprint level are contained in a transcription factor. The new transcription factor which combines with Above DNA and controls an imprint by the approach of this invention can be isolated. the approach (the volume for Hiroto Okada, a "new cell technology experiment protocol", and Shujunsha -- in 1993) that above-mentioned detection and screening are well-known to this contractor The volume for Takaaki Tamura, and "biotechnology manual series 5 transcription-factor approach", Yodosha and 1993, Inouye and C.et al., DNA Cell Biol., 13, and 731-742 (1994) Reference, for example, the approach using an affinity column, a south western blotting method, the foot printing method, and EMSA (electrophoretic mobility shift assay) -- law and one-hybrid -- it can carry out by law etc. Moreover, it can also carry out in the interaction analysis between DNA protein by the joint measurement using surface plasmon resonance using a DNA fixed bead etc., such as pulldown assay and BIACORE, or fluorescence polarization measurement etc.

[0025] For example, when using the affinity column method, a nucleus extract etc. can be applied to the column which fixed DNA of this invention in sepharose or a latex bead as a specimen, and the protein combined using DNA fixed in the column and DNA which has the same array can be eluted after washing a column.

[0026] By the EMSA method (called the gel shifting method), Indicator DNA and the DNA affinity factor (usually nucleus extract of a cell) which usually consist of a 2 chain oligonucleotide of several 10 bases are made to react, and the DNA binding protein contained in a nucleus extract is combined with DNA. Then, if electrophoresis is performed using non-denaturalizing polyacrylamide gel, since the mobility of DNA which protein combined will become smaller than the mobility of uncombined DNA, it is the approach of carrying out using DNA which carried out the indicator of this, and detecting the mobility. As a nucleus extract of a cell, the nucleus extract of a culture Homo sapiens mesangial cell can be used, for example.

[0027] A completely complementary oligonucleotide is compounded to each other, it considers as a single strand in 95-degree-C 5 minutes during a heat block, and a heat block is turned off after that, and specifically, it leaves as it is until it returns to a room temperature (formation of 2 chains of a single stranded DNA). For example, 32P label is performed using MEGALABEL(trademark) Kit (Takara brewing company), and ProbeQuant(trademark) G-50 Micro Columns (Pharmacia) etc. refines (indicator of DNA). a reaction -- for example, -- The nucleus extract containing the protein of 10microg is added 10% 1 mM EDTA 1 mM DTT 10 mM Tris-Cl (pH7.5) under existence of Glycerol, 1mM MgCl2, 0.15MKCl, Salmon Sperm DNA(denatured) 1microg, and Salmon Sperm DNA(non-denatured)1microg, and it is made to react in ice for 15 minutes. If required, DNA (for example, 10pmol) which has not carried out a label as non-indicator competition TITA will be put in here. Add DNA which carried out the label (for example, about 80000cpm), it is made to react at a room temperature further for 20 minutes, and electrophoresis is carried out by polyacrylamide gel 4.5%. After drying gel, a pattern is analyzed with autoradiography. Thereby, the nucleoprotein combined with a probe can be checked.

[0028] DNA which contains the same array as an indicator probe or other transcription factor junction sequences as non-indicator competition TITA can be used. as a transcription factor junction sequence -- for example -- Joint motifs, such as AP-1, AP-2, SP-1, oct-1, and NF-kappaB, can be used. If a band shift disappears by these competition TITA, it will be suggested that the same transcription factor as combining with competition TITA had combined with the probe. Moreover, identification of the protein combined with a probe can also be carried out for example, by super shift assay. For example, if the antibody to a known transcription factor is added to reaction mixture and a super shift is seen, it turns out that the transcription factor which the added antibody combines forms complex with a probe.

[0029] The detection approach of this invention can be used for inspection and the diagnosis of the abnormalities of gene expression in which DNA of this invention participates. Since it is thought that the variation of DNA of this invention, the variation of a transcription factor, or the abnormalities in a manifestation cause a critical heredity disease, these inspection and diagnoses can be performed by the above-mentioned detection approach using DNA of this invention. That is, the protein combined with DNA of this invention in a specimen is detected by the above-mentioned approach. If abnormalities are in association with the amount of the protein combined with DNA of detected this invention, structure, or DNA of this invention and this protein, it will be suggested that abnormalities are in the gene imprint

through DNA of this invention. Especially this inspection or diagnosis is effective in inspection of the abnormalities of MEGSIN gene expression accommodation, and is applied to inspection of the kidney disease especially accompanied by the abnormalities in MEGSIN gene expression. For example, mesangial cell fecundity kidney disease is mentioned to such a disease.

[0030] Moreover, in order to screen the protein combined with DNA of this invention, a south western blotting method can be used. For example, mRNA to cDNA of the cell (for example, mesangial cell etc.) origin expected that the transcription factor combined with DNA of this invention is discovered is prepared. Then, the cDNA library which included this cDNA, the expression vector 11, for example, lambdagt, of Escherichia coli, is produced, a fusion protein with the beta-galactosidase can be made to be able to compound, this fusion protein can be made to be able to stick to a nitrocellulose membrane, DNA of this invention by which the indicator was carried out with radioisotope can be used as a probe, and the phage which compounds a fusion protein with avidity can be detected or chosen.

[0031] Moreover, if a footprinting method is used, the DNA array which the protein combined with DNA of this invention combines can be specified. The junction sequence of the protein combined with DNA of this invention can be identified by specifically using as a probe DNA of this invention which carried out the indicator with radioisotope, and digesting and carrying out electrophoresis of this by DNase I after specimens, such as a nucleus extract, and a reaction.

[0032] When using the one-hybrid method, DNA of this invention is inserted in the upstream of a reporter gene, and a reporter stock is created by incorporating in genomes, such as yeast. next -- the above -- cDNA -- GAL -- four (DNA affinity transcriptional activator of yeast) -- activation -- a domain (GAL4 AD) -- a coding region -- connecting -- making -- these -- a fusion protein -- a code -- carrying out -- as -- activation -- a domain -- (-- AD --) -- a library -- producing -- the reporter stock of the above-mentioned [ this ] -- introducing . When hybrid protein with the protein in which Above cDNA carries out a code to AD combines with DNA of this invention, an imprint is activated and the effectiveness can be detected through the manifestation of a reporter gene. In this approach, it is desirable to use DNA which a CTGATTCAC part contains two copies, three copies, or more than it, using the array which arranged DNA of this invention in the tandem two copies, three copies, or more than it.

[0033] By detection and the screening approach of this invention, the transcription factor combined with DNA of this invention can be identified or isolated. This invention offers the transcription factor which can be identified or isolated by the detection approach of above-mentioned this invention, or the screening approach. Moreover, the detection and the quantum of a

transcription factor which are combined with DNA of this invention by this detection approach are possible. That is, existence of this transcription factor and an amount can be determined for the complex of a transcription factor and DNA of this invention detection and by carrying out a quantum. Moreover, this detection can estimate the abnormalities of the affinity of DNA of this invention, and a transcription factor, or the size of this transcription factor can also be measured through gel electrophoresis etc. by it. Moreover, this invention offers the binder of DNA containing the DNA binding protein which can be identified or isolated by the detection approach of above-mentioned this invention, or the screening approach of this invention. These binders are useful to screening of the drugs which can adjust association with DNA of this invention etc.

[0034] Moreover, in above-mentioned detection and screening, same detection may be performed using the contrast recombinant DNA which made CTGATTCAC suffer a loss, and the result may be compared. Namely, this invention is the detection approach of the compound combined with DNA of this invention at a CTGATTCAC dependence target. (a) The process which contacts the sample containing a sample compound to DNA containing CTGATTCAC of this invention, (b) In the process and the (c) process (a) of making DNA to which the sample containing a sample compound was set to this DNA, and it has varied [ CTGATTCAC is missing or ] contacting, and (b), association with each DNA and this compound is detected, and it is related with an approach including the process which compares both association. The effectiveness of CTGATTCAC exerted on association of the compound combined with DNA of this invention and it by this approach is detectable. That is, if the association detected at the process (a) is intentionally stronger than association detected in the process (b), it turns out that this compound is combined with DNA of this invention at the CTGATTCAC dependence target. Moreover, this invention is the approach of screening the compound combined with a CTGATTCAC dependence target to DNA of this invention. (a) The process which contacts the sample containing a sample compound to DNA containing CTGATTCAC of this invention, (b) In the process and the (c) process (a) of making DNA to which the sample containing a sample compound was set to this DNA, and it has varied [ CTGATTCAC is missing or ] contacting, and (b) Detect association with each DNA and this compound, and it compares with association to the process which compares both association, and DNA to which (d) this CTGATTCAC is missing or has varied. It is related with an approach including the process which chooses the compound intentionally combined strongly to DNA containing this CTGATTCAC. Deletion of the CTGATTCAC is carried out, for example, or DNA which permuted CTGATTCAC by other arrays (for example, CaGAaTCtC; base to which the small letter was mutated) as DNA which

made CTGATTCAC suffer a loss is mentioned. Or when two or more CTGATTCAC(s) are included, the copy number can also use little DNA. By these approaches, the compound specifically combined to CTGATTCAC can be chosen more certainly.

[0035] If the detection approach of this invention is used, the effectiveness of a sample compound exerted on association with DNA of this invention and the protein combined with this DNA can be detected, or the compound which adjusts this association can be screened. This invention is the approach of evaluating the effectiveness of a sample compound exerted on association with the protein combined with DNA and this DNA of this invention. (a) The process at which this DNA and this protein are contacted under existence of the sample containing a sample compound, (b) It is related with an approach including the process which evaluates the effectiveness of the sample compound which exerts the process and (c) this association which detect association with this DNA and this protein on this association by comparing with association with this DNA and this protein under the nonexistence of a sample compound. If it compares under the nonexistence of a sample compound and the joint level of the this DNA and this protein under existence rises, this sample compound has the effectiveness which promotes this association, and if the joint level of the this DNA and this protein under this sample compound existence falls, it will be judged that this sample compound has the effectiveness which controls this association. Moreover, if this detection approach is used, it is possible to screen the compound which adjusts association with the protein combined with DNA and this DNA of this invention. Namely, this invention is the approach of screening the compound which adjusts association with the protein combined with DNA and this DNA of this invention. (a) The process at which this DNA and this protein are contacted under existence of the sample containing a sample compound, (b) the process and (c) this association which detect association with this DNA and this protein by comparing with association with this DNA and this protein under the nonexistence of a sample compound It is related with an approach including the process which evaluates the effectiveness of a sample compound exerted on this association, and the process which chooses the compound which adjusts association with (d) this DNA and this protein.

[0036] The protein combined with DNA of this invention used by the above-mentioned approach may be purification protein, or may be un-refining or rough purification protein. For example, you may be the gestalt of the nucleus extract of a cell etc. For example, the protein refined from the nucleus extract of human cells, such as HDF (human dermal fibroblast: Homo sapiens skin fibroblast), HRE (human renal epithelial cell: Homo sapiens kidney epithelial cell), HMC (human mesangial cell: Homo sapiens mesangial cell), HeLa, floor line, Chang liver, or A431 cell, or a mammalian cell or there

can be used suitably. Preparation of the nucleus extract from a cell can be performed according to Dignam's and others approach (Dignam and J.D.et al., Nucl.Acid Res., 11, and 1475-1489 (1983)). Moreover, separation and purification of the protein from a nucleus extract can be carried out with the well-known biochemical model containing a salting-out, gel filtration, affinity chromatography, ion exchange chromatography, etc. Moreover, manifestation screening of the protein combined with DNA of this invention as mentioned above using mRNA prepared from these cells can be performed, and cloning of the gene which carries out the code of the protein combined with DNA of this invention can be carried out. The obtained clone can be made to be able to discover by Escherichia coli, yeast, the insect cell, or the mammalian cell, and recombination protein can be prepared. In this way, the obtained recombination protein can be used for the approach of above-mentioned this invention. Moreover, the known transcription factor combined with DNA of this invention may be rearranged, and you may prepare, or receive and use as protein.

[0037] As protein combined with DNA of this invention, the protein of the request combined with a CTGATTCAC dependence target is used for DNA of this invention, for example. AP-1 is mentioned as such protein. In AP-1 (activator protein 1), it is one of the main transcription factors of a vertebrate, and is protein complex including the member of a Jun family, and/or the member of a Fos family. c-Jun, JunB, and JunD are contained as a member of a Jun family. Moreover, as a member of a Fos family, c-Fos, FosB, and Fra1 and Fra2 are contained. The hetero or gay dimer with which a Jun family and a Fos family are both formed through a leucine zipper including a basic amino acid cluster and a leucine zipper (bZIP) adjusts the imprint of the gene which combines with DNA and is down-stream. AP-1 used in the approach of this invention may be the mixture of two or more sorts of complex with which Jun to constitute differs from a Fos family, and a specific Fos/Jun heterodimer may be used for it. AP-1 used in this invention -- desirable -- AP-1 of mammalian -- it is Homo sapiens AP-1 more preferably.

[0038] This invention is the approach of evaluating the effectiveness of a compound exerted on association with DNA of this invention, and AP-1. (a) The process at which this DNA and AP-1 are contacted under existence of the sample containing a sample compound, and (b) this DNA -- this -- this [ association / which detect association with AP-1 / the process and (c) this association ] DNA under the nonexistence of a sample compound -- this -- it is related with an approach including the process which evaluates the effectiveness of a sample compound exerted on this association by comparing with association with AP-1. If it compares under the nonexistence of a sample compound and the joint level of this DNA under existence and

AP-1 rises, this sample compound has the effectiveness which promotes this association, and if the joint level of this DNA under this sample compound existence and AP-1 falls, it will be judged that this sample compound has the effectiveness which controls this association. Since it is possible to control the imprint of a gene by adjusting association with DNA of this invention and AP-1, this detection approach is useful also in order to detect the compound which adjusts the imprint of a gene. Moreover, this invention is the screening approach of a compound of adjusting association with DNA of this invention, and AP-1. (a) The process at which this DNA and AP-1 are contacted under existence of the sample containing a sample compound, (b) -- this DNA -- this -- this [ association / which detect association with AP-1 / the process and (c) this association ] DNA under the nonexistence of a sample compound -- this -- by comparing with association with AP-1 the process and (d) this DNA which evaluate the effectiveness of a sample compound exerted on this association -- this -- it is related with an approach including the process which chooses the compound which adjusts association with AP-1. It is useful in order that this screening approach may also screen the compound which adjusts the imprint of a gene. AP-1 complex may be formed of the intracellular autogenous manifestation, can introduce into a cell the gene which carries out the code of the member of for example, a Fos family and a Jun family, and can make it form extrinsically by intracellular [ this ].

[0039] There is especially no limit in the sample compound used for screening or detection in this invention, for example, an inorganic compound, an organic compound, nature or a synthetic saccharide, a peptide, a polynucleotide, protein, nature or a synthetic low molecular weight compound, nature or a synthetic high polymer, an organization or a cell extract, the culture supernatant of a microorganism, vegetation, the natural component of the marine organism origin, etc. are mentioned. Moreover, a manifestation product or a manifestation cDNA library of a gene library etc. can also be used. Before a sample compound contacts the protein combined with DNA and this DNA of this invention, it can be applied to coincidence or the back, and all of these modes are contained in this invention. There is especially no limit in the application approach of a sample compound, and if it is in vitro, it can add to reaction mixture. Moreover, when screening by the system using a cell, it can add to the culture medium of a cell, or transfection can be carried out to intracellular. Moreover, when making protein etc. into a sample compound, a cell may be made to introduce and discover the gene which carries out the code of this protein. A sample compound can be suitably applied as a constituent. For example, it may be mixed with water, a physiological saline, the buffer solution, a salt, a stabilizer, a preservative, suspension, etc.

[0040] If association with DNA and binding protein of this invention falls

under existence of a sample compound compared with the case under the nonexistence of a sample compound, this compound will serve as a candidate of a compound who has the activity which checks this association. Moreover, if this association is promoted by the sample compound, this compound will serve as a candidate of the compound which promotes this association. These compounds are useful as drugs which adjust the imprint by DNA of this invention.

[0041] Moreover, DNA of above-mentioned this invention can be used in order to control an imprint. This invention offers the approach of controlling the imprint including the process which inserts or removes DNA of this invention in the imprint regulatory region of a desired gene of this gene. Moreover, the manufacture approach including the process which inserts or removes DNA of this invention in the imprint regulatory region of a desired gene of a recombinant DNA that the transcriptional control of this gene was changed is also included in this approach. The imprint regulatory region of a gene means a DNA field including the array which controls the imprint of a gene, and, generally it exists in the upstream of the transcription initiation site of a gene. An imprint is controllable by inserting or removing DNA of this invention here. Since DNA of this invention can guide an imprint in the specific cell containing a mesangial cell, it becomes possible by inserting DNA of this invention, or changing this invention by base substitution etc., so that DNA may be constituted to make organization unique targets, such as kidney mesangium, discover a foreign gene of it. One copy is sufficient as the copy number of DNA of this invention to insert, or it may be inserted 2 or more ******s, for example, three copies, or more than it. Moreover, when DNA of this invention is contained in the imprint regulatory region of the target gene, transcriptional control can be changed by carrying out deletion of this or permuting by other arrays. In this case, CTGATTCAC in DNA of this this invention It is desirable to make an array suffer a loss.

[0042] Moreover, this invention offers the recombinant DNA by which the gene was functionally connected with the lower stream of a river of DNA of above-mentioned this invention. That DNA and this gene of this this invention have combined this recombinant DNA in the natural condition is DNA combined with a different gestalt. If specified more, that they have joined together in the natural condition artificially will mean DNA combined with a different gestalt. A recombinant DNA is producible by carrying out ligation for example, of the DNA fragments. For example, a recombinant DNA can be manufactured by combining the DNA fragment generated by restriction enzyme processing, PCR magnification, etc. by DNA ligase. It says that DNA of this invention is connected with this gene so that down-stream gene expression can be controlled [ "it was connected functionally" and ]. A lower stream of a river means 3' side of the chain containing CTGATTCAC

of DNA of this invention. Although there is especially no limit in the gene made to connect, the desired gene which the mammalian of Homo sapiens and others has, for example is mentioned. It is possible to make the gene of the request of those other than MEGSIN discover under MEGSIN and similar transcriptional control, using DNA of this invention. This recombinant DNA may include a desired promotor, a desired enhancer sequence, etc. Such a recombinant DNA has transcriptional activity and guides the imprint of the gene connected with this functional target in a suitable cell. The recombinant DNA of this invention has the transcriptional activity depending on CTGATTCAC more preferably. Specifically, it can check that CTGATTCAC contributes the array of CTGATTCAC, or its part to transcriptional activity in the recombinant DNA containing DNA of this invention when it is made to vary and transcriptional activity falls [ deletion or ] intentionally by saying contributing to the transcriptional activity of the gene by which CTGATTCAC was connected down-stream as having the transcriptional activity depending on CTGATTCAC. Although especially the type of deletion and variation is not limited, if it is deletion, specifically, carrying out deletion of nine bases of CTGATTCAC or the part of those will be mentioned. Moreover, if it is the variation by base substitution, permuting all the two bases or three bases of combination of one of 1 of the base of the underline section of CTGATTCAC bases by other bases, for example will be mentioned, and specifically permuting by CaGAaTCtC (base to which the small letter was mutated) etc. will be mentioned (refer to example). If it is multiple copy **** about a CTGATTCAC array, when it is made to vary and transcriptional activity falls [ deletion or ] intentionally one or more in it (or all), this recombinant DNA will be judged to have the transcriptional activity depending on CTGATTCAC. Transcriptional activity can introduce a recombinant DNA into a cell, and can measure it. For example, transcriptional activity is measured in cells, such as a Homo sapiens mesangial cell (HMC). The recombinant DNA which shows the transcriptional activity depending on CTGATTCAC is contained in this invention including the transcriptional control DNA of this invention.

[0043] DNA which inserted the short DNA fragment (for example, 15 - 200 bp extent) of this invention containing CTGATTCAC in the transcription initiation site upstream of the expression vector which contains a desired promotor and a desired gene as such a recombinant DNA, for example is mentioned. This contractor is usually performing such a chimera promotor's production. The short DNA fragment containing CTGATTCAC may introduce only one copy, and may carry out multiple copy (2 or more ******s) installation. For example, it may also be possible to insert DNA of this invention containing 2, 3, or CTGATTCAC of 4 or more ******s, and to acquire the stronger transcriptional control effectiveness. the between to the

latest CTGATTCAC array from a transcription initiation site -- for example, it takes for 80-120bp 50 to 150 bp preferably 30 to 200 bp. In the meantime, a TATA box [Groudine, M.et al., Mol.Cell.Biol., 1, and 281-288] (1981) and/or CAAT box [Maniatis, T.et al., Science, 236, and 1237-1245] (1987) may be contained. The recombinant DNA of above-mentioned this invention contains a TATA box and CAAT box more preferably including a TATA box between a CTGATTCAC array and a transcription initiation site.

[0044] Such a recombinant DNA is very useful as an expression vector. For example, in order to make a desired gene discover in specific cells, such as a mesangial cell, the vector which combined with the upstream of this gene DNA of this invention shown above is built, and this is introduced into a target cell. Thereby, according to DNA of this invention, gene expression is controllable. Especially DNA of this invention has the activity which makes high rate discover a gene with the kidney (mesangial cell). Therefore, DNA of this invention is applicable to development of the expression vector which made the kidney (especially mesangial cell) the target. The recombinant DNA which an imprint activates on such a kidney unique target can be used for vector production for the gene therapy of for example, a kidney disease. Moreover, also in other cells (organ) which discover the transcription factor which guides the imprint through DNA of this invention, the same effectiveness is expectable. General genetic manipulations, such as construction of a recombination vector and installation to the cell of this vector, can be performed in reference (J.Sambrook and D.W.Russell eds., "Molecular cloning: a laboratorymanual", 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001) according to the conventional method of a publication.

[0045] The recombinant DNA of this invention may be included in the suitable vector. When aiming at gene therapy, as a vector used, the vector originating in a retrovirus, a herpes simplex virus, a cytomegalovirus, the Epstein-Barr virus, a bovine papilloma virus, adenovirus, an adeno-associated virus, a Sindbis virus, poxvirus, etc. is mentioned, for example. Moreover, liposome pharmaceutical preparation, such as reconstruction liposome incorporating temperature sensitivity liposome, blood Nakayasu quality liposome, cationic liposome, pH susceptibility liposome, and the envelope protein of a virus, HVJ(Sendai Virus)-liposome The membrane fusion liposome pharmaceutical preparation which gave the membrane fusion ability of viruses, such as [T.Nakagawaet al., Drug Delivery System, 11, 411] (1996), and VSV(vesicular stomatitis virus)-liposome (JP,11-187873,A), can also be used. As a cell used as the object which introduces these vectors, although a mesangial cell, a tubular cell, a macrophage, a lymphocyte, an endothelial cell, a tumor cell, etc. are mentioned, it is not limited to these, for example.

[0046] Moreover, it is possible to evaluate the effectiveness of a sample

compound exerted on the transcriptional activity of DNA of this invention using the above-mentioned recombinant DNA. This approach is an approach including the process which evaluates the effectiveness of a sample compound exerted on this transcriptional activity under existence of the sample containing the (a) sample compound by comparing with the manifestation of the recombinant DNA of above-mentioned this invention under the nonexistence of a sample compound the process which makes the recombinant DNA of above-mentioned this invention discover and the process which detects (b) this manifestation, and (c) this manifestation. Moreover, the compound which adjusts the transcriptional activity of DNA of this invention using this approach can be screened. This screening approach is an approach including the process which evaluates the effectiveness of a sample compound which exerts on this transcriptional activity, and the process which choose the compound which adjusts (d) this manifestation by comparing with the manifestation of the recombinant DNA of above-mentioned this invention under the nonexistence of a sample compound the process which makes the recombinant DNA of above-mentioned this invention discover under existence of the sample containing the (a) sample compound, the process which detect a (b) this manifestation, and a (c) this manifestation.

[0047] The expression vector which inserted DNA of this invention is specifically introduced into a cell, and the gene connected with the lower stream of a river of DNA of this invention is made to discover. This gene expression from an expression vector is detected under existence of a sample compound. As a cell used, HDF, HRE, HMC, HeLa, floor line, Chang liver, or A431 cell is mentioned, for example. In addition, cells, such as a tubular cell, a macrophage, a lymphocyte, an endothelial cell, and a tumor cell, may be used. A mesangial cell is used preferably. Like the above, there is especially no limit, for example, an inorganic compound, an organic compound, nature or a synthetic saccharide, a peptide, a polynucleotide, protein, nature or a synthetic low molecular weight compound, nature or a synthetic high polymer, an organization or a cell extract, the culture supernatant of a microorganism, vegetation, the natural component of the marine organism origin, etc. are mentioned by the sample compound used for screening. Moreover, a manifestation product or a manifestation cDNA library of a gene library etc. can also be used. There is no limit in the timing which applies a sample compound. For example, when using cell lineage, the sample containing a sample compound can be added to culture medium etc. Moreover, a gene is introduced into a cell and it is good also considering the manifestation product as a sample compound. If manifestation level rises by the sample compound, this compound will become the candidate of a compound who raises the transcriptional activity of DNA of this invention.

Moreover, if manifestation level falls by the sample compound, this compound will become the candidate of a compound who reduces the transcriptional activity of DNA of this invention.

[0048] The existence of promotor activity and the strength of promotor activity can be judged by combining with the lower stream of a river of DNA of this invention the gene (reporter gene) which generally carries out the code of the protein which can perform a quantum easily by the luminous reaction, color reaction, etc., introducing this into a host cell, and detecting color reaction and luminescence. Promotor activity is detected under existence of the sample containing a sample compound, and promotor activity is influenced by the sample compound, or the extent is measured. Compared with the case under the nonexistence of this sample compound, the compound which raises the promotor activity as drugs with which the compound to which promotor activity is reduced checks the transcriptional activity of DNA of this invention is useful as drugs which promote the transcriptional activity of DNA of this invention. Moreover, the transcription factor which activates an imprint through DNA of this invention can also be identified or isolated by above-mentioned detection and screening.

[0049] Moreover, in above-mentioned detection and screening, same detection may be performed using the contrast recombinant DNA which made CTGATTCAC suffer a loss, and the result may be compared. Namely, this invention is the approach of detecting the effectiveness of a sample compound exerted on the transcriptional activity of DNA of this invention. (a) The process which makes the recombinant DNA which includes Above CTGATTCAC under existence of the sample containing a sample compound discover, (b) In the process, the (c) process (a), and each of (b) which make the recombinant DNA to which it set to this DNA and it has varied [ CTGATTCAC is missing or ] under existence of the sample containing a sample compound discover By detecting the manifestation of each DNA and comparing this manifestation with the manifestation of each of this DNA under the nonexistence of the sample compound in each CTGATTCAC of a process and (d) this sample compound which evaluates the effectiveness of the sample compound in the transcriptional activity of each of this DNA --- it is related with an approach including the process which detects specific effectiveness. Moreover, this invention is the approach of screening the compound which adjusts the transcriptional activity of DNA of this invention. (a) The process which makes the above-mentioned recombinant DNA which contains CTGATTCAC under existence of the sample containing a sample compound discover, (b) In the process, the (c) process (a), and each of (b) which make the recombinant DNA to which it set to this DNA and it has varied [ CTGATTCAC is missing or ] under existence of the sample containing a sample compound discover By detecting the manifestation of

each DNA and comparing this manifestation with the manifestation of each of this DNA under the nonexistence of the sample compound in each the process which detects specific effectiveness and CTGATTCAC(e) CTGATTCAC of a process and (d) this sample compound which evaluate the effectiveness of the sample compound in the transcriptional activity of each of this DNA -- it is related with an approach including the process which chooses the compound which adjusts an imprint specifically. That is, the compound which adjusts an imprint on a CTGATTCAC unique target can be obtained by setting at a process (e) and choosing the compound which adjusts this manifestation with strong extent intentionally to the manifestation of DNA containing CTGATTCAC as compared with the effectiveness given to the manifestation of DNA to which CTGATTCAC is missing or has varied. At a process (c), it can ask for the ratio (change multiple) to the manifestation level under the nonexistence of the manifestation level under existence of a sample compound, for example in DNA containing CTGATTCAC, and DNA which is not included. In a process (e), the change multiple in DNA containing CTGATTCAC chooses a compound which is more nearly intentionally [ than the change multiple in DNA which does not contain CTGATTCAC ] expensive. Thus, the compound to which the compound to which the manifestation of DNA containing CTGATTCAC is reduced with the selected compound reduces the manifestation of DNA of this invention, and the compound raised conversely are judged to be the compound which raises the manifestation of DNA of this invention. As DNA which made CTGATTCAC suffer a loss, DNA to which deletion of the CTGATTCAC was carried out, or DNA permuted by other arrays (for example, CaGAaTCtC; base to which the small letter was mutated) is mentioned, for example. Or when two or more CTGATTCAC(s) are included, the copy number can also use little DNA. Or another promotor may be used. By these approaches, the compound which acts specifically to CTGATTCAC can be chosen more certainly.

[0050] The compound which may be chosen by the screening approach of this invention is useful as a transcriptional control agent (an imprint accelerator or imprint inhibitor). Moreover, this invention offers an imprint inhibitor including the isolation DNA including DNA of this invention, or the base sequence which at least 15 bases which contain CTGATTCAC of the base sequence of a publication in array number:1 more preferably followed. For example, by medicating a cell, such DNA can check an operation of a transcription factor as a decoy nucleic acid, and can control an imprint. Moreover, if the Mie chain structure is made to form in promoterregion, association to the promotor of a transcription factor can be checked. Especially the transcriptional control agent of this invention is useful as a transcriptional control agent of a MEGSIN gene. This invention relates to the

activity as a transcriptional control agent of the compound which may be chosen by DNA of this invention, and screening of this invention, and the activity in manufacture of a transcriptional control agent. Moreover, this invention relates to the method including the process which prescribes for the patient the compound which may be chosen by DNA of this invention, and screening of this invention of adjusting an imprint. The transcriptional control agent of this invention turns into drugs which adjust growth of a mesangial cell. That is, the drugs which promote the imprint of a MEGSIN gene turn into an accelerator of growth of a mesangial cell, and the drugs which control the imprint of a MEGSIN gene turn into an inhibitor of growth of a mesangial cell. Discovering a MEGSIN gene by the kidney mesangial cell, self-possessed sthenia of the immune complex which changes from an immunoglobulin or complement to the hyperplasia of the Tsuguaki cell proliferation which makes a mesangial cell a subject, and a mesangium substrate, and a list is accepted, and sthenia of the manifestation causes the symptom of typical mesangial proliferative glomerulonephritis (WO 01/24628). Moreover, that the amount of MEGSIN manifestations is accelerating in a IgA glomerulonephritis patient or a diabetic nephropathy patient also supports that sthenia of a manifestation of MEGSIN is participating in the onset of kidney disease. Thus, especially the drugs that control the imprint of a MEGSIN gene are useful as a remedy to mesangial cell fecundity kidney disease. The mesangial cell fecundity kidney disease in this invention means the kidney disease accompanied by growth of a kidney mesangial cell, and the hyperplasia of a mesangium substrate. IgA glomerulonephritis, the membranoproliferative glomerulonephritis, an SLE (systemic lupus erythematosus) nephropathy, diabetic nephropathy, or a cryoglobulin nephropathy is contained in such kidney disease. For example, by prescribing the imprint inhibitor of this invention for the patient, it is thought possible to control the onset and progress of such mesangial cell fecundity kidney disease. That is, this invention relates to the preventive and the remedy of mesangial cell fecundity kidney disease which make an active principle the transcriptional control agent of this invention which controls the imprint of the MEGSIN gene of this invention. Moreover, this invention relates to the activity of the transcriptional control agent of this invention for manufacture of the preventive of mesangial cell fecundity kidney disease, or a remedy.

[0051] Moreover, on the other hand, since it is the protein belonging to a SERPIN super family, as for Homo sapiens MEGSIN, a possibility of causing the thromboembolism by sthenia of blood coagulation ability or the hemorrhagic disease by sthenia of fibrinogenolysis ability also has Homo sapiens's MEGSIN abnormalities (Suzuki et al., protein, a nucleic acid and an enzyme, 34 volumes, and 949-962 (1989)). This suggests that the drugs which affect the transcriptional activity of DNA of this invention may act on

the onset and control of these diseases. Therefore, the utilization as a remedy [ as opposed to the thromboembolism and a hemorrhagic disease in the drugs in which screening of this invention is useful also in order to obtain the drugs relevant to these diseases, and it is chosen, and deals ] is also expected.

[0052] The candidate compound chosen by the screening approach of this invention can be used as the object for the therapy for mesangial cell fecundity kidney disease, and/or the principal component of the remedy constituent for prevention after examining safety, stability, etc. further. The remedy constituent of this invention can be pharmaceutical-preparation-ized according to a well-known galenical pharmacy-manufacturing method, and can be prescribed for the patient. Moreover, the compound itself which is a principal component can also be directly prescribed for the patient. When pharmaceutical-preparation-izing, a medicine can be prescribed for the patient, combining suitably the medium or support generally used as drugs.

[0053] Moreover, if the code of this compound is carried out by DNA and it gets, this DNA will be included in the vector for gene therapies, and performing gene therapy will also be considered. Administration can be performed by approaches, such as intraarterial injection, an intravenous injection, the administration in a nasal cavity, the administration in a bronchial tube, permucosal administration, dermal administration, intramuscular administration, hypodermic administration, internal use, intrarectal administration, and direct administration to the affected part. A dose is changed according to conditions, such as a medication method, whenever healthy, a patient's weight, age, and, but if it is this contractor, it can choose a suitable dose suitably.

[0054] That is, for example in a mesangial-proliferative-glomerulonephritis model animal (WO 01/48019), effective concentration is determined by comparing the relaxation effect of a nephritis symptom among various doses. And a dose to which the concentration of the administration compound in a mesangial cell reaches the effective concentration by each above administration roots is determined experientially. In a general administration gestalt, the dose per weight of 1kg is determined as that from which an active principle is distributed over the whole body. If it is the compound considered that kidney translatability is high based on the analysis result of the pharmacokinetics and metabolism in a laboratory animal, a dose can be set up lower.

[0055] The remedy constituent of this invention is blended with a medium or support in consideration of the dose and administration gestalt which were determined. This contractor is usually performing blending an active principle so that a required dose can be attained. More generally the active principle can set 1micro [ of usual / per weight of 1kg ] g-50mg of doses of the

remedy constituent by this invention to 10micro g-10mg (for example, 10micro g-1mg). Moreover, in the case of injections, about [ of internal use ] 1/100 can be made into the rule of thumb of a dose. A dose can be adjusted by performing a still more nearly special dosage form design. For example, in such pharmaceutical preparation, although it can also consider as gradual release-ized pharmaceutical preparation by holding to suitable support, since the remedy constituent of this invention can maintain high blood drug concentration, it can set up loadings low.

[0056] As a pharmaceutical form in the case of administering orally, there are powder, a granule, a capsule, a pill, a tablet, elixirs, suspension, an emulsion, syrups, etc., and it can choose suitably. Moreover, gradual-release-izing, stabilization, ******-izing, formation of difficulty breaking, enteric-izing, easy absorption-ization, etc. can be embellished about these pharmaceutical preparation. Moreover, as a pharmaceutical form in the case of performing oral cavity internal division place administration, there are a peptizing agent, a hypoglottis agent, a buccals, trochiscus, an ointment, a application-with-gauze agent, liquids and solutions, etc., and it can choose suitably. Moreover, gradual-release-izing, stabilization, ******-izing, formation of difficulty breaking, enteric-izing, easy absorption-ization, etc. can be embellished about these pharmaceutical preparation.

[0057]

[Example] Hereafter, this invention is not restricted by these examples although an example explains this invention concretely. In addition, all the reference quoted by this description is incorporated as some of these descriptions.

[0058] [Example 1] The genome DNA fragment to upstream region abbreviation-4.0kbp of a Homo sapiens MEGSIN gene was newly isolated from isolation of the imprint regulatory region of a Homo sapiens MEGSIN gene, and the identification BAC clone of a transcription initiation site, and since the DNA array in connection with transcriptional activity was specified, this genomic DNA array was determined (array number: 1). Moreover, in order to determine a transcription initiation site with an exact Homo sapiens MEGSIN gene, the five prime end of MEGSIN mRNA by the primer extension method was identified. total RNA was extracted from the Homo sapiens mesangial cell (BioWhittaker) cultivated by the Dulbecco alteration Eagle's medium (DMEM) containing 10% fetal calf serum (GIBCO), the penicillin of 100 IU/mL, the streptomycin of 100microg/mL, and the L-glutamine of 200microg/mL. T four The indicator DNA probe was created using the polynucleotide kinase. Namely, it is presumed that transcription initiation site order is fully covered. It is DNA about an oligonucleotide primer (5 '-AGGCTGTCCA AAGGTGCAGC -3' / array number: 3) (it became clear that it dealt with +67-+86). A five prime end indicator kit (MEGALABEL

(trademark): TAKARA SHUZO) is used. After carrying out a five prime end label by [gamma-32P] ATP, gel filtration chromatography (Sephadex(trademark) G-50-harmacia company) refined the resultant, and it was used as an isotope indicator primer.

[0059] Next, 1xTE and 0.25M KCl After incubating at 60 degrees C for 1 hour, at the room temperature, total RNA of 10microg prepared from the indicator primer and the culture Homo sapiens mesangial cell under existence was left for 1 hour and a half, and carried out annealing. To a DNA-RNA hybrid, 5xfirst strand buffer 16microL, 0.1M DTT 8microL, 2.5M dNTP 8microL, and d-H2O 28microL and 1micro (SUPERSCRIPT II(trademark):GIBCO BRL) of reverse transcriptase L are added. After incubating for 1 hour and making it elongate at 37 degrees C, ethanol precipitate of the resultant is carried out. After dissolving in dye and incubating at 100 degrees C for 5 minutes, leave it for 5 minutes and it was made to denaturalize in Hikami, and it added to 8% acrylamide gel created beforehand, electrophoresis was performed, gel was dried, and autoradiography analyzed. Moreover, the same primer performed the sequence reaction (deltaTth DNA Polymerase SequencingPRO: Toyobo) for the genomic DNA which contains a MEGSIN promotor simultaneously, and the transcription initiation site of MEGSIN mRNA was determined by comparing by performing migration ( drawing 1 ).

[0060] Consequently, it became clear that the 1st exon of a Homo sapiens MEGSIN gene was 373bp(s). That is, the transcription initiation site was the 4023rd T of array number:1. Moreover, the TATA box [Groudine, M.et al., Mol.Cell.Biol., 1, and 281-288] (1981) existed in the part of upper abbreviation 35bp of a transcription initiation site.

[0061] [Example 2] The vector which combined the upstream region (BamH I-Xba I fragment; -4021bp-+130bp) of a MEGSIN gene, or its deletion variation DNA and luciferase gene was produced for assay of the functional assay imprint regulatory region of MEGSIN imprint regulatory region. recombination of the restriction enzyme site where various deletion variation DNA exists in DNA -- or the field used for assay -- a wrap -- the primer pair [ like ] was designed and it prepared by polymerase chain reaction (PCR). It specifically cut to the Stu I site of an upstream region -2542, the Sac I site of -1874, the PmaC I site of -1451, the Kpn I site of -1052, etc., and when required, it was made the blunt end, and it inserted by ligation into the following luciferase expression vector, and produced. Moreover, the used primer is as follows although these constructs were produced by the PCR method to mold about the construct of -1052 or less bps.

- 834:5'-TAGGTACCAGGTGTAGGCAACCAACTGG-3' (array number: 4)
- 504:5'-TAGGTACCGCAGTACAAAGAGAAGCCAG-3' (array number: 5)
- 240:5'-TAGGTACCCAGAAGAGTATGTTTTGACC-3' (array number: 6)

- 72:5'-TAGGTACCGATACTATTTTGAAACCTGG-3' (array number: 7)
In addition, all antisense primers used the following primers.
Antisense: 5'-TAGATCGCAGATCTCGAGCCCCTAGAC-3' (array number: 8)
[0062] These PCR products were cut by Kpn I and BgI II (or Xho I), and were built into this site of a luciferase expression vector (pGL3-Basic Vector:Promega). The deletion variation DNA created by the above approach is a MEGSIN gene, respectively. It is -4021-+130, -2542-+130, -1874-+130, -1451-+130, -1052-+130, -834-+130, -504-+130, -240-+130, and -72-+130. These constructs were introduced to the cell according to the attached description using the transfection reagent (LipofectAMINE PLUS(trademark):GIBCO BRL).
[0063] First, the vector incorporating the various deletion variation DNA was introduced to Hominoidea epidermis cancer cell stock A431 cell, and it examined what array performs forward or negative control in this promoterregion ( drawing 2 A). After carrying out transfection of the vector on the conditions of 3 hours or 6 hours by 37 degrees C and cultivating for one day or two days at 37 degrees C, removed culture medium, washed the cell once by PBS, it was made to dissolve by the lysis (Cell lysis) buffer, and rye SETO was obtained. The amount of luminescence of fluorescence was measured directly using RUMINO meter (Lumat LB9507 tube luminometer(trademark):Berthold Technologies). Relative luciferase activity was searched for by **(ing) with the measured value of the Idemitsu force by pRL-CMV [ KOTORANSUFEKUSHON / pRL-CMV ].
[0064] Furthermore, same examination was carried out to the example 1 using the culture Homo sapiens mesangial cell (BioWhittaker) of a publication ( drawing 2 B). Consequently, it has checked that the pattern of transcriptional activity which the pattern of transcriptional activity and A431 cell which a culture Homo sapiens mesangial cell shows show was the same ( drawing 2 A and B). Moreover, there is no bigger fluctuation to transcriptional activity in the construct between -240-+130 than -4021-+130, and it became clear that transcriptional activity was decreasing sharply between -240-+130 and -72-+130. It became clear that the same transcriptional control as the culture Homo sapiens mesangium was performed from these things to a MEGSIN promotor also in A431 cell in which discovering MEGSIN like a culture Homo sapiens mesangial cell is known. Moreover, it became clear that the imprint regulatory region which shows a positive regulation very powerfully among -240--72 in this promotor array existed.
[0065] next -- the construct of -4021-+130 -- using -- HUVEC (normal Homo sapiens umbilical cord intravenous hide cell), HDF (normal Homo sapiens skin fibrocyte) and HMC (normal Homo sapiens mesangial cell), and list floor line (Homo sapiens amnion origin cell strain), HeLa (Homo sapiens endometrium origin cell strain), and A431 -- and -- Transfection was carried

out to Chang Liver (Homo sapiens hepatic-carcinoma origin cell strain), and luciferase activity was measured. Consequently, in the Homo sapiens normal cell, strong transcriptional activity was seen in HMC ( drawing 3 A). Moreover, also in the Homo sapiens origin cell strain, transcriptional activity comparatively strong against A431 was seen ( drawing 3 B), and it became clear that the used imprint regulatory region had cell singularity.

[0066] - As a result of producing a short construct to 10 bp unit between 179-+130 and -72-+130 and examining transcriptional activity using A431 cell, sharp decrease of transcriptional activity was seen between -121-+130 and -99-+130 ( drawing 4 ). - the place which searched the motif between 121--99 -- AP-1, oct-1, and TCF-11 etc. -- the array of a high score was found ( drawing 5 ). while producing the construct to which deletion of the about 55 base sequence bp including the array concerned was carried out -- AP-1 and oct-1 -- or -- TCF-11 As a result of producing a construct including each variation ( drawing 6 ) and examining transcriptional activity using A431 cell like the above, in the construct (-85 - -139 deletion structure) to which deletion of the 55 bp was carried out, transcriptional activity fell thoroughly ( drawing 7 ). Moreover, by AP-1 mutant, lowering of about 50% of transcriptional activity was seen to fluctuation not being looked at by transcriptional activity in oct-1 mutant ( drawing 7 ). Furthermore, when same examination was performed using A431 cell about the construct to which deletion only of the part of an AP-1 joint motif was carried out, lowering of about 50% of transcriptional activity was seen like the mutant ( drawing 8 ).

[0067]

[Effect of the Invention] By this invention, the transcriptional control DNA of the promotor origin of the MEGSIN gene specifically discovered to the mesangial cell was offered. DNA of this invention -- a mesangial cell -- it can use as an element of the promotor for specific gene expression, for example, the application to the gene therapy of various kidney disease can be considered. Moreover, it is also possible to screen protein, such as a transcription factor combined with this DNA, using DNA of this invention. Furthermore, this DNA is applicable also to screening of the drugs which control MEGSIN gene expression.

[0068]

[Layout Table]

SEQUENCE-LISTING<110> KUROKAWA, Kiyoshi MIYATA, Toshio<120> Transcription regulatory-elements-and their use<130> KRK-A0104<140><141><160> 13 <170> PatentIn Ver. 2.0<210> 1<211> 4230<212> DNA<213> Homo sapiens<400> 1ggatcctggt ggctcccagt ttttattctc aggttttact tttattttat atcttattta 60 taatgattga tagaattgta aatgaaatga ccacaataag ataaactttt aatagtttcc 120 taatcttatt agacttccca ttagcttata

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[Translation done.]

## DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing the identification result of the MEGSIN transcription initiation site by the primer extension method.
[Drawing 2] It is drawing showing the transcriptional activity pattern of the deletion mutant of the MEGSIN promoterregion in A431 cell (A) and a Homo sapiens mesangial cell (HMC) (B). - The array which just controls an imprint exists in the field of 240 to -72.
[Drawing 3] It can set to various normal Homo sapiens (primary culture) cells (A) and a Homo sapiens origin cell strain (B). -4021--130 It is drawing showing the cell singularity of the imprint regulatory region contained in a construct.
[Drawing 4] It is drawing showing the transcriptional activity of the deletion mutant of MEGSIN promoterregion. - The array which just controls an imprint exists in the field of 121 to -99.
[Drawing 5] It is drawing showing the transcription factor junction sequence found out by a MEGSIN promotor's field of -121 to -99. The array in drawing is equivalent to 3893-3939 of array number:1.
[Drawing 6] It is drawing showing the structure of a MEGSIN promotor's imprint regulatory region. The array in drawing is equivalent to 3761-4230 of array number:1. - The deletion field of 85 - -139 deletion structure was illustrated. Moreover, the variation array when mutating the junction sequence of AP-1, oct-1, and TCF-11 was shown below.
[Drawing 7] It is drawing showing the transcriptional activity of various variants.
[Drawing 8] It is drawing showing the transcriptional activity of the deletion mutant of AP-1 junction sequence.

[Translation done.]